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Pluronic Triblock Copolymer Systems and Their Interactions with Ibuprofen

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Small-angle neutron scattering and pulsed-field gradient stimulated-echo nuclear magnetic resonance (NMR) have been used to study the structural characteristics of aqueous Pluronic solutions. In particular, changes in the micellar structure upon addition of ibuprofen to the solutions and altering the temperature have been investigated. Increases in temperature and ibuprofen concentration both appear to favor micellization, with increases observed in the aggregation number, fraction of polymer micellized, and core radius of the micelle, along with a decrease in the volume fraction of the solvent in the core. At high drug concentrations and/or temperatures, micelles of the more hydrophobic Pluronics scatter neutrons strongly at low Q, indicating attractive interactions between micelles or a change in the shape of the aggregates. The addition of ibuprofen to Pluronic P104 has also been found to reduce the critical micellization temperature from approximately 20 °C to below 13 °C. The hydrophobicity of the Pluronic, quantity of ibuprofen present, and temperature of the solution all seem to have a strong influence on the extent and nature of aggregation observed.

1. Introduction

Over recent years, there has been significant interest in the study of block copolymers, in particular, regarding their potential as drug-delivery agents. Pluronic triblock copolymers consist of a central poly(propylene oxide) (PPO) block with terminal poly(ethylene oxide) (PEO) blocks and tend to micellize in aqueous solution because of the hydrophobicity difference between the PPO and PEO blocks. These micelles contain a hydrophilic corona of PEO and a hydrophobic core of PPO,[1,2] within which drugs can be solubilized and transported, creating potential for more effective controlled release in the body.[3]

Pluronics often display complex phase behavior, which is influenced by the number of PEO and PPO blocks present in the polymer. Increasing the size or PPO content of a Pluronic has been shown to cause reductions in both its critical micelle concentration (cmc) and critical micellization temperature (cmt),[4] and the micellar structures of different Pluronics are well-characterized.[5,6] Temperature and concentration are also key factors in the phase behavior,[7,8] and many recent studies have focused on the gel states formed by Pluronics at high concentration and temperature.[9,10]

In addition to the ability of Pluronics to solubilize and stabilize drugs, there is also evidence that they are able to enhance the effect of certain drugs (such as chemotherapeutics[11−13]) by sensitizing specific biological cells. The choice of Pluronic for optimum delivery appears to depend upon the drug involved,[14] and there is now a wealth of literature on the drug solubilization and release properties of Pluronics. However, relatively few studies have investigated the change in micellar structure of Pluronic micelles on the uptake of drug molecules. This is crucial in understanding the release profiles *in vivo*, because the size and aggregation number of the micelles is critical in determining blood circulation times and bioavailability of the drug,[1,15] as well as the maximum amount of drug that can be solubilized.

The sensitive nature of Pluronic micellization means that the addition of other compounds is likely to alter the aggregation characteristics; indeed, it has been found that the addition of co-solvents or solutes to aqueous Pluronic solutions can influence properties, such as the cmt and cmc.[16−19]

Sharma et al. have recently investigated the structural effects of adding pharmaceuticals to Pluronic F127, using small-angle neutron scattering (SANS), dynamic light scattering, and UV spectroscopy.[10,20,21] They found some drugs to increase the cmc of F127 and others to decrease it, with no clear correlation to the characteristics of the drugs. However, an increase in the core and corona size was noted upon addition of a wide range of drugs,

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as well as a general decrease in the aggregation number and polydispersity. The gelation boundaries were observed to decrease upon addition of drugs, in agreement with the findings of Scherlund et al., who solubilized anesthetic drugs using F127 and F68.[22]

In this paper, we describe the use of small-angle neutron scattering to investigate the structural changes in a series of Pluronics, upon addition of increasing amounts of the nonsteroidal anti-inflammatory drug ibuprofen. Pluronics P103, P104, and P105 were chosen, which each contain a similar number of PPO units but increasing PEO content, as outlined in Table 1. The effect of the temperature on the structures has also been considered, by studying P104 samples with and without ibuprofen at a range of temperatures. Determination of properties, such as the aggregation number, micelle size, fraction of polymer micellized, and solvation of the core, using a range of different polymer compositions and temperatures, should provide insight into the polymer−drug interactions and potential drug-release characteristics of the polymers.

2. Materials and Methods

2.1. Materials. All compounds were used as received. Pluronics P103, P104, and P105 were provided by BASF. Ibuprofen, 40 grade, was supplied by Albemarle. D_2O, 99.94 at % D, was purchased from Goss Scientific Instruments Ltd. Matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI−TOF MS) measurements were carried out to confirm the composition of the polymers, and the values determined are presented in Table 1.

2.2. Preparation of Solutions. All samples were prepared by weighing the Pluronic and solvent into a vial, sonicating for approximately 1 h, and leaving on a roller-mixer for 24 h to equilibrate. The ibuprofen was then added to the solutions, and samples were sonicated for a further 2 h to allow for incorporation of ibuprofen into the micelles. Samples were left on a roller-mixer again for at least 24 h to reach equilibrium.

2.3. MALDI−TOF MS. All experiments were performed using an Applied Biosystems 4700 proteomics analyzer (TOF/−TOF), with a 200 Hz Neodinium YAG laser (operating at 355 nm). The spectrometer was operated in positive-ion reactor mode, with the laser intensity set just above the ionization threshold. The mass range was set to between 1000 and 10 000 Da, depending upon the nature of the sample. A total of 50 aquisitions with 25 laser shots each were summed for each sample, and external calibration was carried out prior to running the samples. Measurements were carried out on samples using tetrahydrofuran (THF) as the solvent, NaCl as the counterion, and dithranol as the matrix.

2.4. SANS. The SANS measurements were carried out on the NG7 SANS3 instrument at the National Institute of Standards and Technology (NIST) Center for Neutron Research, Gaithersburg, MD. Two configurations were used: one with the detector offset by 25 cm and a sample−detector distance of 1 m and the second with the detector offset by 10 cm and a sample−detector distance of 13 m, to give a scattering vector (Q) range of 0.005−0.600 $Å^{-1}$. Cold neutrons with a wavelength of 6.0 Å were used, and all samples were measured in 1 mm path-length demountable titanium cells. Unless otherwise stated, samples were measured at 293 K. The raw data were reduced using Igor Pro macros.[23]

Data were fitted using the Pedersen model for Pluronics in solution,[24,25] which uses a form factor based on a dense spherical core surrounded by polymer chains, which display Gaussian statistics, as shown in Figure 1. This model allows unimers and micelles to coexist, accounting for the gradual aggregation characteristics of Pluronics, and has previously been used to fit scattering data from Pluronics.[25,26]

Table 1. Composition of Pluronics Used

	P103	P104	P105
ethylene oxide units[a]	34	54	74
propylene oxide units[a]	60	61	56
M_n (g mol^{-1})[b]	4686	5866	6228
M_w (g mol^{-1})[b]	5408	6386	6900
polydispersity index[b]	1.15	1.09	1.11

[a] Values supplied by BASF. [b] Determined using MALDI−TOF MS.

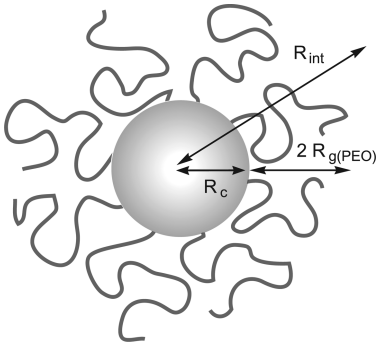

Figure 1. Schematic of the Pedersen model for block copolymer micelles, showing the core radius (R_c), interaction radius (R_{int}), and radius of gyration of the PEO segment ($R_{g(PEO)}$).

The form factor, F_{mic}, given in eq 1, combines four different terms: the self-correlation of the sphere [$F_s(Q, R_c)$], the self-correlation of the chains [$F_c(Q, L, b)$], the cross term between the sphere and the chains [$S_{sc}(Q)$], and the cross term between chains [$S_{cc}(Q)$]. These parameters have previously been described fully by Pedersen and Gerstenberg.[24]

$$F_{mic}(Q) = (N_{agg})^2\beta_s{}^2F_s(Q, R_c) + N_{agg}\beta_c{}^2F_c(Q, L, b) + N_{agg}(N_{agg}-1)\beta_c{}^2S_{cc}(Q) + 2(N_{agg})^2\beta_s\beta_cS_{sc}(Q) \quad (1)$$

where R_c is the radius of the core, L and b represent the contour length and the Kuhn segment length of the PEO block of the polymer, and β_s and β_c are the excess scattering lengths of the polymer blocks in the sphere (PPO) and chain (PEO), respectively, calculated by multiplying their respective scattering densities (ρ_s and ρ_c) by the volume of the chain.[27] The form factor was modified to account for micellar polydispersity, assuming a Gaussian distribution of the aggregation number N_{agg}.

The form factor for scattering from the unimers, F_{uni}, follows the Debye function, as described in eq 2

$$F_{uni}(Q) = \frac{2(\exp(-x)-1+x)}{x^2} \quad (2)$$

where $x = (QR_g)^2$ and R_g is the unimer radius of gyration.

The form factor and structure factor [$S(Q)$] were combined as shown in eq 3 to give the scattering intensity.[27] This takes into account the fact that the micelles are not centrosymmetric, because of differences in polymer chain configurations at the surface of the micelle. The structure factor derived by Lekner for monodisperse hard spheres was used,[28] and an allowance for the resolution of the instrument was included by convoluting the

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fit with a simple Gaussian function[29]

$$\frac{\partial \Sigma}{\partial \Omega}(Q) = N[F_{\mathrm{mic}}(Q) + A_{\mathrm{mic}}^{\mathrm{av}}(Q)^2(S(Q)-1)] \qquad (3)$$

where N is the number density of the micelles and $A_{\mathrm{mic}}^{\mathrm{av}}(Q)$ is the Fourier transform of the average radial scattering length density distribution for each micelle.

The model used incorporates the following fitting parameters: aggregation number, concentration, volume fraction of solvent in the core, radius of gyration of the PEO segments and unimer, respectively, volume fraction of hard spheres, interaction radius of hard spheres, polydispersity of the micellar aggregation number, fraction of polymer micellized, Q resolution, scattering length density of the solvent, and background scattering. The large number of fitting parameters was necessary because of the polydispersity of the Pluronics and the complexity of their aggregation behavior.[25] However, the high number of parameters does mean that it is possible to obtain many different fits of the data to the model. To maintain consistency between data sets and avoid "over-fitting" the data, the maximum possible number of parameters was fixed, including concentration, Q resolution, and scattering length density of the solvent, leaving only seven parameters that were allowed to vary for each sample. The radii of gyration for both the PEO segments and the unimer were initially allowed to vary and then were fixed at the average values found for each series of samples. The fitted models for data discussed in this paper give reduced χ^2 values between 1 and 18.

2.5. Pulsed-Field Gradient Stimulated-Echo Nuclear Magnetic Resonance (PFGSE−NMR). PFGSE (diffusion) NMR measurements were carried out at 293 K on a Bruker DSX-300 MHz spectrometer with a diff 30 field gradient probe using a 5 mm ^{1}H/^{2}H coil insert. The gradient pulse duration (δ) was set to 1 ms, and the magnetic field gradient (G) ramped from 0.05 to 10 T m^{-1}. The diffusion time, Δ, was set between 50 and 250 ms, depending upon the sample. To maximize the signal/noise ratio, 64 scans were accumulated over at least 50 gradient steps. A calibration of the instrument was carried out using a water/methanol reference sample. Further information on the PFGSE−NMR data analysis can be found in ref 30.

3. Results and Discussion

Solutions of P103, P104, and P105 without drug were all studied initially at 5 wt %/vol concentration, 293 K, as shown in Figure 2. At this composition and temperature, it is clear that P103 shows considerably more aggregation than P104 and P105, reacted in the stronger intensity of scattering at low Q. This is unsurprising, because the more hydrophobic nature of P103 would render it the least stable of the three in aqueous solution and our data correlates with findings by Alexandridis et al., who reported a small increase in cmc with the size of the PEO blocks.[4] Nolan et al. have also observed P104 and P105 to display similar aggregation to one another at 25 °C, with P103 significantly more micellized.[5] In fact, our fits to the Pedersen model reveal that P103 has N_{agg} = 34.8 at 20 °C (Table 2), whereas P104 and P105 samples show only a few unimers per micelle; this is likely to be around the onset of aggregation. (It should be noted that slightly different figures for the aggregation number can be obtained by altering the fit to the Pedersen model; however, in all cases, the trends shown by the parameters are the same.) Pluronics tend to micellize over a slightly broad temperature/concentration range, which is a reaction of their inherent

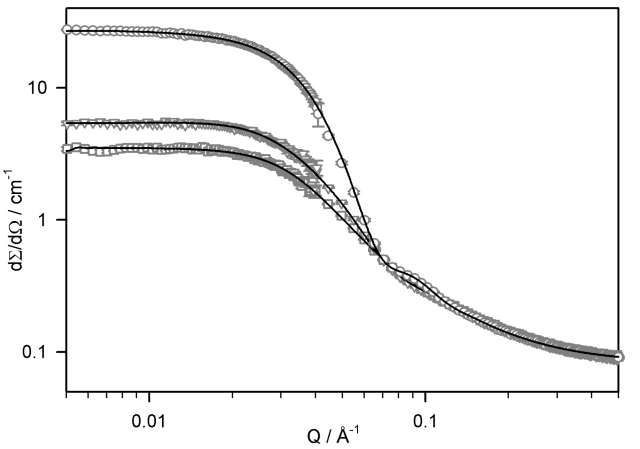

Figure 2. SANS from a series of 5 wt %/vol Pluronics in D_2O, equilibrated at 293 K: (○) 5 wt %/vol P103, (▽) 5 wt %/vol P104, and (□) 5 wt %/vol P105. Solid lines are fits to the Pedersen model for Pluronic triblock copolymers. Error bars representing one standard deviation are smaller than the plotting symbols.

Table 2. SANS Parameters for a Range of Pluronics at 5 wt %/vol Concentration, at 293 K[a]

Pluronic	P105	P104	P103
aggregation number (N_{agg}, ±0.9)	2.9	5.1	34.8
volume fraction of solvent in the core (±0.01)	0.93	0.91	0.69
volume fraction of hard spheres (±0.01)	0.03	0.03	0.08
interaction radius of hard spheres (±5.3, Å)	110.4	112.4	66.1
relative polydispersity of N_{agg} (±0.03)	0.30	0.40	0.60
fraction micellized (±0.01)	0.71	0.70	0.68
core radius (±0.6, Å)	38.2	43.0	53.5
PEO R_g (Å)	15.7	13.4	12.5
unimer R_g (Å)	23.3	21.4	20.0

[a] Data fitted to the Pedersen model for block copolymers. Errors represent one standard deviation.

polydispersity. The P103 sample also shows a side maximum, as expected to result from the form factor of dense spherical objects with a sharp interface (in this case, the core−corona interface between PPO and PEO).[31]

The SANS fits show that the aggregation number of the polymeric micelles at 20 °C increases with an decrease in the polymer size and hydrophilicity, and the volume fraction of solvent in the core decreases correspondingly as the micelles become better formed. The core radii can be calculated from the aggregation number and fraction of solvent in the core according to eq 4; these are 54, 43, and 38 Å for P103, P104, and P105, respectively

$$R_c = \left[\left(\frac{3}{4\pi}\right)\frac{N_{\mathrm{agg}}N_{\mathrm{PO}}V_{\mathrm{PO}}}{1-\phi_{\mathrm{sol}}}\right]^{1/3} \qquad (4)$$

where R_c is the core radius, N_{PO} is the number of propylene oxide units per chain, ϕ_{sol} is the volume fraction of solvent in the core, and V_{PO} is the volume of one propylene oxide unit, 9.63×10^{-29} m^3 (calculated from the density).

The fraction of polymer micellized actually appears to be marginally higher for the more hydrophilic polymers; however, this is likely to be a consequence of the fact that the aggregation number is very low for these polymers. Thus, a group of just 2−3 polymer chains together could be classified as micellized.

Upon addition of ibuprofen, all three systems showed dramatic changes (Figure 3), with similar trends observed for the different

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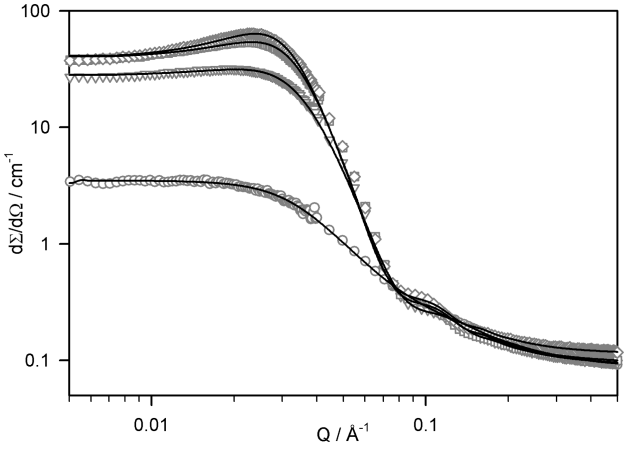

Figure 3. SANS from 5 wt %/vol Pluronic P105 in D_2O with increasing quantities of ibuprofen, equilibrated at 293 K: (○) 0.00 wt %/vol ibuprofen, (▽) 0.25 wt %/vol ibuprofen, (□) 0.50 wt %/vol ibuprofen, and (◇) 0.75 wt %/vol ibuprofen. Solid lines are fits to the Pedersen model for Pluronic triblock copolymers.

Table 3. SANS Parameters for 5 wt %/vol P105 with Increasing Ibuprofen Concentration, at 293 K[a]

ibuprofen concentration (% wt/vol)	0.00	0.25	0.50	0.75
aggregation number (N_{agg}, ±3.3)	2.9	48.5	87.8	99.6
volume fraction of solvent in the core (±0.01)	0.93	0.42	0.17	0.12
volume fraction of hard spheres (±0.01)	0.03	0.12	0.16	0.18
interaction radius of hard spheres (±9.3, Å)	110.4	84.1	88.6	93.3
relative polydispersity of N_{agg} (±0.09)	0.30	0.45	0.48	0.50
fraction micellized (±0.01)	0.71	0.73	0.78	0.80
core radius (±4.5, Å)	38.2	47.6	51.4	52.6

[a] Data fitted to the Pedersen model for block copolymers. The R_g values for the PEO segment and unimer were fixed at 15.7 and 23.3 Å, respectively.

Pluronic samples. In all cases, a significant increase in the aggregation number was observed, along with an increase in the fraction of polymer micellized (Table 3). This is in contrast to studies of F127 with the addition of a range of drugs, in which aggregation numbers are reported to decrease,[20,21] and indicates that the presence of ibuprofen drives aggregation of the block copolymers. The volume fraction of hard spheres increases accordingly, as does polydispersity of the micelles around the onset of micellization. However, the volume fraction of solvent in the core shows a considerable decrease, suggesting the uptake of ibuprofen and a concomitant change in the micelle structure toward more compact, better formed micelles, with reduced solvent penetration into the PPO core. An overall increase in micellar size was confirmed by diffusion NMR data, as shown by the attenuation plot of P104 with and without ibuprofen (Figure 4). These results indicated a hydrodynamic radius of 86 Å for P104 micelles in a solution of 0.5 wt %/vol ibuprofen, which is significantly higher than the radius of gyration calculated from SANS results, on account of the micelle corona and extent of hydration contributing to the hydrodynamic radius.[32]

Assuming that the majority of the ibuprofen is located inside the micelle (because of its poor water solubility), this will also contribute to the scattering observed. The number of ibuprofen molecules per micelle can be calculated from the SANS parameters and reaches a maximum of around 500 for the P105 sample with 0.75 wt %/vol ibuprofen. However, modification of the

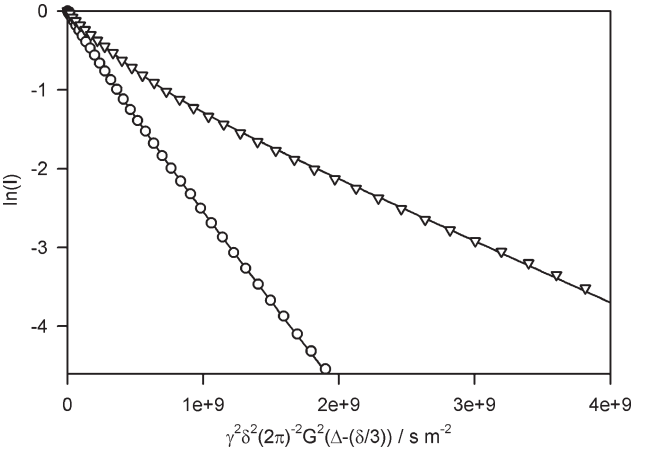

Figure 4. Attenuation plot from diffusion NMR data, of Pluronic P104 in D_2O at 293 K: (○) 5 wt %/vol P104 and 0.00 wt %/vol ibuprofen and (▽) 5 wt %/vol P104 and 0.50 wt %/vol ibuprofen. All data are taken from the attenuation of the Pluronic CH_2 group peak at $\delta \sim 3.3$ ppm.

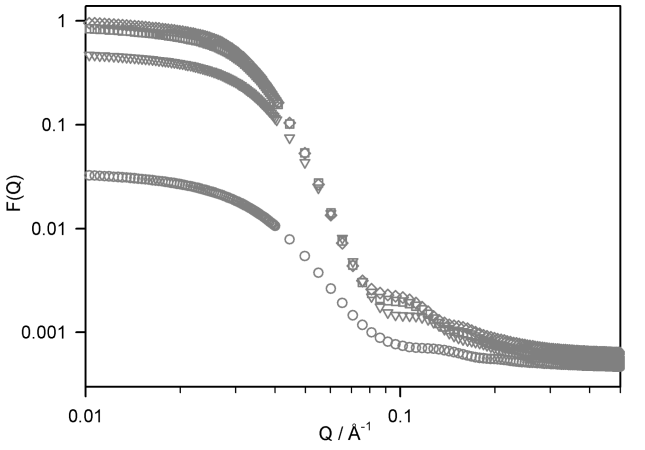

Figure 5. Form factor [$F(Q)$] for 5 wt %/vol Pluronic P105 in D_2O with ibuprofen at 293 K, extracted from the fit shown in Figure 3: (○) 0.00 wt %/vol ibuprofen, (▽) 0.25 wt %/vol ibuprofen, (□) 0.50 wt %/vol ibuprofen, and (◇) 0.75 wt %/vol ibuprofen.

fitting model to incorporate a scattering element from ibuprofen in the micellar core was found to have little influence on the overall fit. The modification involved addition of parameters to define the volume fraction of ibuprofen in the micelle core and the scattering length density of the ibuprofen. The volume fraction of ibuprofen was added to the volume fraction of solvent in the core when calculating the total core volume, and the scattering length density of the core was also adjusted according to the amount of drug present in the core and the scattering length density of the drug. The addition of these extra parameters was found to marginally reduce the volume fraction of solvent in the core determined from the model; however, because the quantities of ibuprofen used were very small, none of the other parameters were affected. Because additional parameters increase the uncertainty in the fitting, this model was not used in the final data fitting; therefore, it is possible that the volume fraction of solvent in the core could be overestimated by up to 0.05 for samples containing ibuprofen.

Extraction of the structure factor and the micellar form factor from the fits clarifies the changes occurring in the polymer system. Figures 5 and 6 show an enhancement of both the form factor and the structure factor intensity as the ibuprofen concentration

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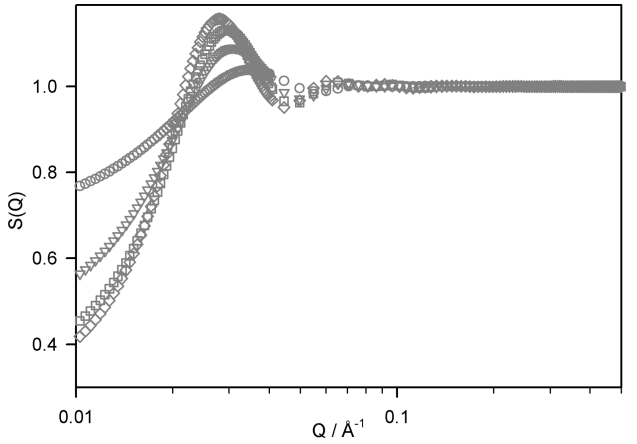

Figure 6. Structure factor [$S(Q)$] for 5 wt %/vol Pluronic P105 in D_2O with ibuprofen at 293 K, extracted from the fit shown in Figure 3: (○) 0.00 wt %/vol ibuprofen, (▽) 0.25 wt %/vol ibuprofen, (□) 0.50 wt %/vol ibuprofen, and (◇) 0.75 wt %/vol ibuprofen.

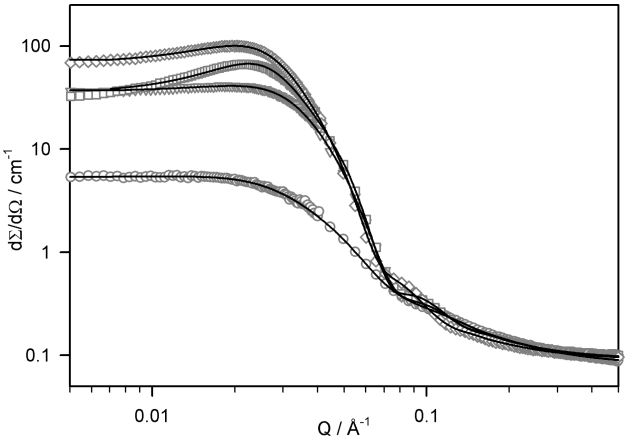

Figure 7. SANS from 5 wt %/vol Pluronic P104 in D_2O with increasing quantities of ibuprofen, equilibrated at 293 K: (○) 0.00 wt %/vol ibuprofen, (▽) 0.25 wt %/vol ibuprofen, (□) 0.50 wt %/vol ibuprofen, and (◇) 0.75 wt %/vol ibuprofen. Solid lines are fits to the Pedersen model for Pluronic triblock copolymers.

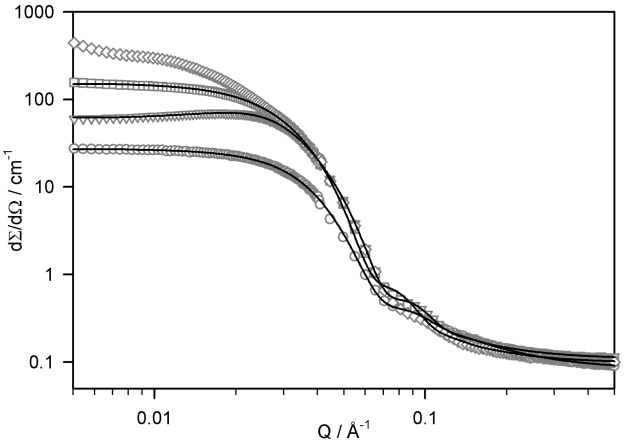

Figure 8. SANS from 5 wt %/vol Pluronic P103 in D_2O with increasing quantities of ibuprofen, equilibrated at 293 K: (○) 0.00 wt %/vol ibuprofen, (▽) 0.25 wt %/vol ibuprofen, (□) 0.50 wt %/vol ibuprofen, and (◇) 0.75 wt %/vol ibuprofen. Solid lines are fits to the Pedersen model for Pluronic triblock copolymers.

is increased, which is a result of the fraction of polymer micellized rising. The reduction of the solvent in the core also contributes to the change in the form factor, because this leads to stronger contrast between the micelle and solvent. The slight shift of the form factor to lower Q values is indicative of the increase in micellar size, while the shift of the structure factor to lower Q values is a result of the increasing distance between micelles. These seemingly contradictory trends occur because, although the fraction of polymer micellized is increasing, this is counteracted by the rise in aggregation number with the ibuprofen concentration, meaning that, overall, there are fewer micelles in solution and the distance between them is greater.

The main distinction between the series of Pluronics with added ibuprofen is that, for the smaller, more hydrophobic Pluronics, at high ibuprofen concentration, the scattering intensity tends to increase more significantly at low Q (Figures 7 and 8). This reduces the effectiveness of the spherical Pedersen model to reproduce the scattering curves observed. There are two possible explanations for the appearance of such a feature: either a change in shape away from the spherical structure that the Pedersen model is based on or an attractive interaction occurring between the micelles. If changes in shape are occurring, the scattering may be modeled more effectively by using the amended models developed by Pedersen et al., which describe scattering from block copolymers that form micelles with ellipsoidal or cylindrical cores.[33] Any attractive interactions present, on the other hand, could potentially be accounted for by the use of a "soft-sphere" structure factor (such as that developed by Hayter and Penfold[34]) in the scattering model, instead of the traditional hard sphere.

The possibility of a change in the shape of the aggregates being formed for these Pluronics has previously been discussed by Nagarajan, who modeled the addition of hydrophobic compounds to a range of Pluronics and predicted the formation of cylindrical and lamellar structures upon the addition of the most hydrophobic compounds to P103 and P104.[35] Guo et al. have also described a change in the shape of P105 aggregates upon the addition of increasing quantities of 1-phenylethanol, from spherical to cylindrical and finally forming disk-like micelles.[36] However, the samples of P103 with 0.50 and 0.75% ibuprofen were observed to phase-separate over time scales of a few weeks; therefore, for these samples, it is likely that the feature can be attributed to attractive interactions between the aggregates.

This interpretation was supported by diffusion NMR results, which showed that, while the micellar species of most samples diffused at around 2×10^{-11} m^2 s^{-1}, relating to a hydrodynamic radius of 9.4 nm, P103 with the highest ibuprofen content showed two separate micellar diffusion rates, the slower of which diffused at around 4×10^{-14} m^2 s^{-1}. Diffusion rates of this order of magnitude indicate the presence of very large aggregates, suggesting that the onset of aggregation/phase separation was already occurring in the system. The presence of separate diffusion coeffcients for micelles and larger species confirms that the aggregation is gradual, with spherical micelles existing at the same time as larger structures. Separate diffusion coefficients were also observed for unimeric and micellar species, indicating that exchange between micelles and solution is slow, as suggested by Malmsten et al.[37]

Because temperature is also a key factor in the aggregation characteristics of Pluronics, two P104 samples, with and without ibuprofen, were investigated at a series of temperatures. For the Pluronic without ibuprofen, at 13−17 °C, the scattering could be

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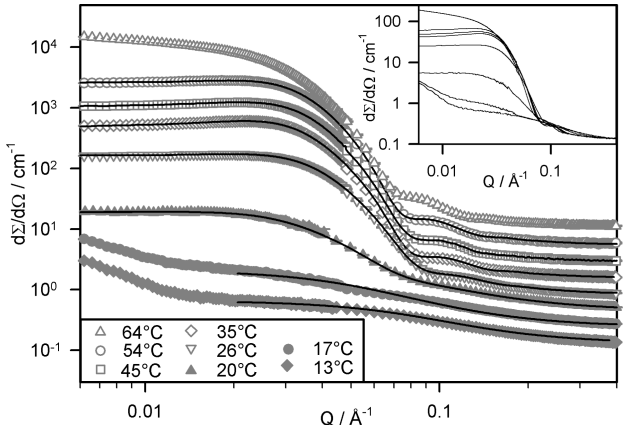

Figure 9. SANS from 5 wt %/vol Pluronic P104 in D_2O, at a range of temperatures. Solid lines are fits to the Pedersen model for Pluronic triblock copolymers. Data have been shifted vertically to see the clarity of fits, and the inset shows the data (in the same order as the main graph) without shifting.

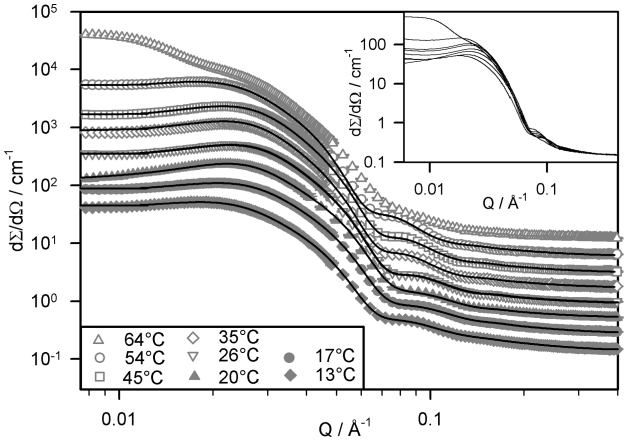

Figure 10. SANS from 5 wt %/vol Pluronic P104 and 0.5 wt %/vol ibuprofen in D_2O, at a range of temperatures. Solid lines are fits to the Pedersen model for Pluronic triblock copolymers. Data have been shifted vertically to see the clarity of fits, and the inset shows the data (in the same order as the main graph) without shifting.

fitted to the Debye function, suggesting that the polymer was largely unimeric. The excess scattering length density was fixed at 5.91×10^{-6} Å^2, and the molecular weight was fixed at 5866 g mol^{-1}, which gave fits with radii of gyration of 22.1 and 27.2 Å for unimers at 13 and 17 °C, respectively. These sizes were confirmed by Guinier analysis, using plots of $\ln(d\Sigma/d\Omega)$ against Q^2, and are also slightly higher than the unimer hydrodynamic radius of 21 Å calculated from diffusion NMR data at 20 °C. Upon heating from 17 to 26 °C, there was a distinct increase in the scattering, indicative of micellization of the Pluronic, as a result of dehydration of the PPO block at high temperatures.[38,39] This cmt correlates with previously reported values of 18[4,39] and 22.3 °C,[40] respectively. (It should be noted that the 20 °C samples shown in Figures 9 and 10 were measured on a different occasion to the other samples (but using the same equipment). To account for the different background observed for this sample, its scattering has been raised by 0.05 cm^{-1}, so that it is comparable to the other samples. The slightly lower scattering observed in the low Q region for this sample (Figure 10) highlights the sensitivity of Pluronic micellisation; even factors such as an exchange of H_2O with D_2O in the sample over time have been known to affect the aggregation.[41])

Table 4. SANS Parameters for 5 wt %/vol P104 at a Range of Temperatures, Fitted to the Pedersen Model for Block Copolymers[a]

temperature (°C)	20	26	35	45	54
aggregation number (N_{agg}, ±0.4)	5.1	29.4	54.6	63.4	74.5
volume fraction of solvent in the core (±0.01)	0.91	0.54	0.37	0.32	0.28
volume fraction of hard spheres (±0.01)	0.02	0.08	0.11	0.11	0.10
interaction radius of hard spheres (±0.5, Å)	112.4	79.2	88.7	86.3	87.8
relative polydispersity of N_{agg} (±0.01)	0.40	0.43	0.36	0.36	0.37
fraction micellized (±0.01)	0.70	0.88	0.99	0.99	0.99
core radius (±0.1, Å)	43.0	44.8	49.5	50.8	52.5

[a] The R_g values for the PEO segment and unimer were fixed at 13.4 and 21.4 Å, respectively.

The gradual increase in scattering observed highlights the broad micellization behavior typical of commercial Pluronics because of their polydispersity. Even at 25 °C, diffusion NMR measurements revealed a wide cmc range: upon varying the concentration of P104 from 1.0 to 5.0 wt %/vol, a steady increase in the proportion of polymer with the slower (micellar) diffusion coefficient was observed, as well as a gradual loss of the hyperfine structure of the PPO CH_2CH signal, which occurs upon micellization.[40]

Upon increasing the temperature further, it was found that similar trends were observed to those upon the addition of ibuprofen: an increase in the aggregation number, fraction micellized, and core radius, and a decrease in the volume fraction of solvent in the micelle core (Table 4). These trends correlate with those previously reported by Joseph et al.[26] for F127, Guo et al.[36] for F84, and Borbély[42] for F68, with increasing temperature. Wanka et al. describe P104 solutions at temperatures ranging from 20 to 45 °C to display aggregation numbers increasing from 1 to 121 and micellar radii from 3.9 to 6.1 nm, as determined by static light scattering.[40] Our sample is of similar size and aggregation number to these at low temperature but appears to aggregate less substantially with the temperature increase, which may be due to differences in the concentration or Pluronic batch. Yang and Alexandridis found 8 wt % P105 to display aggregation numbers rising from 50 to 78 upon heating from 30 to 60 °C;[43] these are very similar to our results, although P104 would be expected to show greater aggregation than P105 because of its increased hydrophobicity.

As can be seen in Figure 9, at 64 °C, the scattering at low Q increases from a Q^0 power law toward a Q^{-1} dependence, which indicates a transition from spherical to cylindrical micelles. This is similar to the change in scattering observed by Aswal et al.[44] for solutions of P85 with varying KCl concentration, and it is probable that, if the temperature were increased further, a linear Q^{-1} dependence at low Q would be observed. The formation of rod-like micelles, followed by a two-phase region, upon an increase of the temperature and/or concentration of a P104 solution, was previously predicted by Linse, who modeled the phase behavior of Pluronics.[45]

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Table 5. SANS Parameters for 5 wt %/vol P104 with 0.5 wt %/vol Ibuprofen at a Range of Temperatures, Fitted to the Pedersen Model for Block Copolymers[a]

temperature (°C)	13	17	20	26	35	45	54
aggregation number (N_{agg}, ±2.3)	58.6	62.2	65.4	87.1	107.4	116.7	140.8
volume fraction of solvent in the core (±0.01)	0.52	0.39	0.28	0.21	0.15	0.10	0.10
volume fraction of hard spheres (±0.01)	0.10	0.12	0.15	0.15	0.15	0.15	0.11
interaction radius of hard spheres (±1.3, Å)	117.8	106.5	107.7	102.4	106.2	105.8	119.4
relative polydispersity of N_{agg} (±0.02)	0.41	0.40	0.48	0.38	0.41	0.43	0.48
fraction micellized (±0.01)	0.77	0.82	0.91	0.92	0.93	0.94	0.92
core radius (±0.5, Å)	55.5	52.3	50.3	53.7	56.2	56.7	60.3

[a] The R_g values for the PEO segment and unimer were fixed at 13.4 and 21.4 Å, respectively.

The P104 sample with ibuprofen responds slightly differently to temperature change. The presence of ibuprofen significantly reduces the cmt, with ibuprofen appearing to act as a "co-surfactant", so that even at 13 °C, the aggregation number is as high as 58 (Figure 10). Again, the aggregation number and fraction micellized increase with the temperature, while the volume fraction of the solvent in the core decreases. The core radius increases with the temperature above 20 °C, although interestingly decreases in size from 13 to 20 °C (Table 5).

Scattering in the low Q region begins to increase at 54 °C for this sample and shows a distinct second length scale upon reaching 64 °C. This is characteristic of a change in shape toward ellipsoidal micelles, as opposed to the cylindrical micelles formed at high temperatures without ibuprofen, meaning that the presence of ibuprofen actually influences the shape of the micelles formed. It also indicates that the temperature at which these structural changes occur is reduced by the presence of ibuprofen in the aggregates. Ellipsoidal Pluronic aggregates were observed by Mortensen and Pedersen[7] for P85 solutions above 65 °C, which were attributed to the PPO block reaching its stretching limit upon heating to these temperatures. Other research groups have ascribed changes in the shape of Pluronic aggregates to a reduction in interfacial tension at high temperatures.[36] It is possible that the presence of ibuprofen in our samples may have either had an influence on the packing of the polymer chains in the micelles, affecting the space available for each unimer, or changed the interfacial tension of the aggregates, altering their preferred surface/volume ratio. Solubilization of the ibuprofen may also require a minimum thickness of the steric stabilizing layer, meaning that ellipsoidal conformations may be favored over cylindrical micelles.

4. Conclusions

SANS and PFGSE−NMR were used to investigate the structural aggregation features of a series of Pluronics with varying ibuprofen content and temperature. It was found that hydrophobicity of the polymer, addition of ibuprofen, and an increase in the temperature all enhanced the tendency of the polymer to aggregate. Similar structural effects were observed in the micelles as a result of altering each of these properties: the aggregation number, fraction of polymer micellized, and micellar size all increased, while the volume fraction of solvent held in the core of the micelle decreased (attributed to the micelles becoming better formed, with less solvent penetration).

For the more hydrophobic Pluronics, the highest temperatures or ibuprofen concentrations tended to cause an increase in scattering at low Q. However, it is thought that this could be attributed to different factors for different samples. At room temperature, P103 containing high concentrations of ibuprofen displayed a gradual phase separation (over a period of weeks) and diffusion NMR confirmed the presence of large aggregates in the samples. It is thought that the increase in low Q scattering for these samples is therefore related to the formation of attractive interactions between micelles. Diffusion NMR confirmed the simultaneous presence of unimers, micelles, and larger aggregates in these solutions, indicating slow exchange of the polymer between all three states.

P104 at raised temperatures showed a more linear gradient at low Q, tending toward Q^{-1}, which suggests a change in shape from spherical to cylindrical micelles. This observation supports previous studies, in which cylindrical or lamellar geometries have been predicted or observed for P103, P104, or P105 Pluronics at high temperature. The addition of ibuprofen to P104 solutions was found to dramatically lower the cmt of the Pluronic, from approximately 20 to below 13 °C. It also influenced the change in shape of the micelles at high temperatures, with SANS data showing a new distinct length scale, indicating the formation of ellipsoidal rather than cylindrical geometries.

These results show that the aggregation behavior of Pluronics P103, P104, and P105 is sensitive to changes in both the temperature of the solution and quantity of ibuprofen present. The addition of ibuprofen to the Pluronic solutions appears to have a similar effect to that of altering the temperature, other than at the highest temperatures and ibuprofen concentrations, where more complex changes in stability and shape of the micelles were observed. Changes in aggregate size and structure are critical in determining the rate of drug delivery from micellar structures; therefore, understanding the changes in these properties could potentially lead to better prediction of polymeric drug-release patterns in the body. However, the sensitivity of micellization shown highlights the difficulty in predicting aggregation characteristics *in vivo*, because of the wide range of properties able to influence the micellization.

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Supporting Information Available: PFGSE−NMR attenuation plots for P103 solutions with the addition of ibuprofen. This material is available free of charge via the Internet at http://pubs.acs.org.